A Modified Indigo Method for the Determination of Ozone in Non-Aqueous Solvents

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Abstract

The indigo method for the analysis of aqueous ozone was modified to allow analysis of dissolved ozone in non-aqueous liquid phases. The method was tested using the solvent decamethylcyclopentasiloxane 245 and a vegetable oil. The molar absorptivity at 600 nm of the indigo trisulphonate molecule was re-checked and found to be 20,069±412 L mol^{-1} cm^{-1} which is in agreement with the generally accepted value. Linear correlation between liquid phase and gas phase ozone concentrations confirmed that ozone solubility in decamethylcyclopentasiloxane 245 obeyed Henry's law with a constant of 1.71±0.09 mg L^{-1} per mg L^{-1} in the gas phase. Ozone solubility in the vegetable oil followed a power law model with $k = 0.148$ and $n = 0.767$ (liquid and gas phase concentrations in mg L^{-1}). The stoichiometry of the reaction between ozone in the non-aqueous phase and

indigo trisulfonate in acidic solution was also confirmed as being about one. Moreover, the reaction products were confirmed by chromatographic analysis. This method was found effective to analyse ozone in non-aqueous solvents with a lower limit of detection of 2.6 µg L^{-1} and upper limit of detection of 142.7 mg L^{-1}.

1. Introduction

The use of ozone in water and wastewater treatment dates back to the end of the 19th century and is now commonplace as it has many advantages over other treatment methods, including being generated *in situ* and normally leaving no residues or by-products except bromate, however there still exist some hurdles which must be overcome if the use of ozone in water treatment is going to continue to grow (Gottschalk et al., 2010). Traditionally, it has been difficult to expose contaminants of water to high levels of ozone due to ozone's relatively poor solubility in water (~0.2 mg L^{-1} per mg L^{-1} in the contacting gas phase at 20°C) and it is still expensive to generate in large quantities (Ward et al., 2003; Ward et al., 2005). It has been proposed that contacting contaminated water with an ozone loaded solvent may serve to increase the extent of degradation of contaminants, by allowing them to contact with more ozone than in ozonated water alone through mass transfer of ozone and/or the contaminants across the liquid-liquid interface, the two immiscible liquids can then be separated by gravity and the solvent reloaded with ozone for reuse. A number of investigations have been carried out in order to observe the effectiveness of this method of contaminant removal by ozone (Chang and Chen, 1994; Bhattacharyya et al., 1995; Guha et al., 1995; Freshour et al., 1996; Ward et al., 2003; Ward et al., 2004; Ward et al., 2005; Gromadzka and Nawrocki, 2006; Ward et al., 2006).

There has also been interest in using ozonated solvents such as vegetable and olive oils for other therapeutic or medical applications; although the behaviour of these “Criegee ozonides” is not fully understood in this context, their highly oxidative nature can be exploited for pathogen destruction with the subsequent generation of oxygen encouraging healing including that of cutaneous wounds (Bocci, 2002; Kim et al., 2009; Travagli et al., 2010).

It has been previously determined that the solvent decamethylcyclopentasiloxane 245 (PMX-0245) is capable of dissolving ten times more ozone than water can when exposed to the gas at a given partial pressure (Ward et al., 2003). This property of the solvent, in addition to its low vapour pressure (< 5.3 mmHg), low toxicity (LD_{50} oral rats = 2 g/kg), low water solubility (17 µg/L) and resistance to ozone attack (Ward et al., 2004) have resulted in the liquid being the solvent of choice for a number of liquid-liquid/ozone experiments carried out by Ward et al.

Ward et al. (2003) were able to determine the concentration of ozone dissolved in samples of PMX-0245 by constantly measuring the concentration of ozone liberated into the gas phase when pure gaseous oxygen was passed through the solvent until the gas phase concentration fell to zero. These measurements were carried out in a closed system and required the use of a data logger in order to accurately record the data over the time period. Although a simple procedure to carry out given the context and setup of the investigation, the procedure does not lend itself well to the batch wise analysis of a sample of PMX-0245 taken from a continuous process due to; a) transferring a sample of solution into the reaction vessel could easily cause turbulence and result in liberation of the dissolved ozone into the open atmosphere, b) the measurements are time consuming and require the integration of a large number of data points, and c) ozone has been found to be stable in the solvent for up to only 10 minutes with

a 50 % decrease in dissolved ozone concentration after 25 minutes (Ward et al., 2003). It is hence desirable to develop a method of analysis which is quick to perform, minimises the risk of liberation of gaseous ozone from the solvent, and is stable for a reasonable amount of time prior to analysis, which minimises ozone loss by decomposition.

Bader and Hoigné (1982) developed the indigo method for the determination of ozone in water. The basis of the indigo method is that ozone will rapidly and stoichiometrically discolour indigo trisulfonate in acidic solution, yielding a linear decrease in absorbance at 600 nm with increasing ozone concentration. The reaction between ozone and acidified indigo trisulfonate solution yields both isatin-5-sulfonic acid and isatin-disulfonic acid as seen in Figure 1 (Bader and Hoigne, 1981) accompanied by a decrease in absorbance at 600 nm as seen in Figure 2. Although under laboratory conditions, the direct UV absorbance of ozone can be used to quantify the dissolved gas, this method cannot be used with great accuracy in real water samples containing organic contaminants, nor in many organic solvents due to the interference caused by the natural UV absorbance of such materials; the interference from these materials at 600 nm is much less (Rakness, 2005). The indigo method has become a popular standard procedure for the batch wise analysis of ozone in water, having the advantages of being easy to carry out, very fast and provides a good balance between cost and selectivity (Clesceri et al., 1998; Gottschalk et al., 2010). In addition, the samples to be analysed via spectrophotometry have been found to be stable for up to 4 to 6 hours after the addition of indigo reagent meaning that measurements of absorbance need not be taken immediately after sampling. Chiou, et al. (1995) expanded on the indigo method developed by Bader and Hoigné (1982) and on other works by Hart et al. (1983) and Collins et al. (1989) by utilising gastight syringes in a similar procedure; the use of gastight syringes

in earlier investigations serving to minimise losses of gaseous ozone during sample transportation and later acting as reaction vessels themselves.

This paper describes a modified indigo method which can be used for the determination of the concentration of ozone in non-aqueous solvents. In this study the solvents decamethylcyclopentasiloxane 245 (PMX-0245) and vegetable oil were used.

2. Method

2.1 Modified indigo method

2.1.1 Principle

Ozone has been shown to rapidly discolour indigo trisulfonate in acidic solution (indigo reagent) linearly with increasing concentration (Bader and Hoigne, 1982). In real water samples, the characteristic absorbance at 600 nm of indigo reagent is easier to measure than the UV absorbance of ozone at 260 nm. Moreover, the extinction coefficient, ε, of indigo at 600 nm, of approximately 20,000 L mol^{-1} cm^{-1}, is much higher than that of ozone at 260 nm, of approximately 2,900 L mol^{-1} cm^{-1}, which increases the sensitivity of the measurement (Bader and Hoigne, 1981). When contacted with the ozonated non-aqueous phase, ozone is transferred to the indigo aqueous phase and acts to discolour the indigo reagent. Being non-miscible phases, the difference in absorbance between an unreacted sample of indigo solution and that of an ozone reacted sample of indigo solution is therefore a suitable basis for determining the concentration of ozone in a non-aqueous sample.

2.1.2 Reagents

The reagents used to develop the modified indigo method for use with non-aqueous solvents were as below. All aqueous solutions were made in Milli-Q water (Millipore Corp.) with

resistivity of 18.2 MΩ.cm. Decamethylcyclopentasiloxane 245 (Dow, UK) and vegetable oil (Costcutter, UK) were used as the non-aqueous solvents. All other reagents were purchased from Sigma Aldrich, UK and were of at least reagent grade.

(i) Indigo Stock Solution (ISS) containing 1 mmol L^{-1} potassium indigo trisulfonate, prepared as described by Bader and Hoigné (1982),

(ii) Indigo Reagent II (IR2) solution prepared as described by Bader and Hoigné (1982),

(iii) Indigo Reagent III (IR3): approximately 25 mL of deionised water was added to a 100 mL volumetric flask. 20 mL of ISS was then added in addition to 0.7 mL concentrated phosphoric acid and 1.3 g of sodium dihydrogen phosphate dihydrate. The flask was made up to the 100 mL mark with deionised water and stored in the dark. The pH of this solution and that of IR2 was measured to be 1.80. IR3 was not used in the development of the indigo method by Bader and Hoigné and had to be developed for use in this modified version; it allows the quantification of concentrations of dissolved ozone which are up to twice as high as IR2 would allow.

The reagents used for the determination of ozone-indigo reaction stoichiometry and by-products were:

(i) 0.2 mmol L^{-1} isatin-5-sulfonic acid in acidic solution: 10 mL of 1 mmol L^{-1} isatin-5-sulfonic acid stock solution was measured and added to a 50 mL volumetric flask. 0.6500 g of sodium dihydrogen phosphate dihydrate was carefully weighed and added to the volumetric flask in addition to 0.350 mL of phosphoric acid. The contents of the flask was then made up to the 50 mL mark with deionised water, shaken together and stored in the dark until use. The pH of this solution was measured to be 1.80.

(ii) 0.2 mmol L^{-1} indigo trisulfonate in acidic solution: as above however, 10 mL of ISS was used instead of 10 mL of 1 mmol L^{-1} isatin-5-sulfonic acid stock solution.

2.1.3 Determination of the extinction coefficient of indigo trisulfonate ε_{ind}

Ten volumes of indigo stock solution ranging in 0.100 mL intervals from 0.100 mL to 1.000 mL were added to 10 mL volumetric flasks and made up to the mark with deionised water. The absorbance at 600 nm of each solution was then measured using a spectrophotometer (Agilent 8543 G1103A) with a quartz cell of path length 1 cm, and plotted against molar concentration of indigo trisulfonate. The value of ε_{ind} was then found from the gradient of this plot. These measurements were performed in triplicate using different batches of indigo stock solution to assess reproducibility.

2.1.4 Open vessel experiments

Initial experiments which required mixing of the two liquid phases in open reaction vessels were found to be highly unreliable and gave rise to irreproducible results. It was decided that in order to minimise off-gassing of dissolved ozone from the PMX-0245, ozone-inert gastight syringes should be used both to sample the ozonated solvent and to act as reaction vessels. The syringe used was an SGE Analytical Gastight, 10 mL syringe with PTFE tipped plunger and push-button valve. The valve served to lock the contents of the syringe inside whilst mixing or standing. All materials in contact with ozone were glass, stainless steel or PTFE.

2.1.5 Spectrophotometric determination of dissolved ozone in non-aqueous solvent

The development of this method was conducted primarily with a focus on the solvent PMX-0245. Due to differences in the behaviours of PMX-0245 and vegetable oil, different volumes of each liquid and different ozonation times were used.

The tare weight of a gastight syringe was found without the needle attached. The needle was only attached to the syringe in order to withdraw samples of indigo solution and non-aqueous liquid phase, weighing of the syringe and dispensing of the samples was carried out without the needle attached in order to avoid weighing and dispensing of an unreacted volume of liquid held up inside the needle. The syringe was then filled with approximately but no less than 6 mL (5 mL for vegetable oil) of IR2 and accurately weighed a second time allowing for determination of the mass of IR2 by difference.

Ozone was generated by a BMT Messtechnik 803 Ozone Generator (BMT-Messtechnik, Germany) from compressed oxygen cylinder (BOC, UK). The primary method of controlling the concentration of ozone in the gas was via the power dial on the ozone generator however, when lower gas phase concentrations of ozone were required, the outlet from the generator was blended with a stream of pure oxygen which served to dilute the gas phase. The gas was directed to a BMT Messtechnik 963 Ozone Analyser (BMT-Messtechnik, Germany) and the concentration of ozone in the gas was recorded, the gas stream was then diverted away from the ozone analyser and through a diffuser into a sample of approximately 40 mL of non-aqueous phase for 20 minutes (1 hr for vegetable oil) with stirring carried out by magnetic mixer and stir bar at 600 RPM. After this time the gas was directed back to the ozone analyser, the concentration of gas phase ozone was recorded a second time and the average of the two readings was taken. Figure 3 shows the apparatus used in this investigation.

After ozonation, a sample of up to 3 mL of ozonated PMX-0245 or 5 mL of vegetable oil was drawn into the syringe, the syringe valve was then closed and the syringe shaken by hand for 30 seconds to ensure good mixing of the two liquid phases. The syringe was then left to stand

upright, with the plunger uppermost, in a suitable vessel to allow the two phases to separate for 5 minutes and then accurately weighed a third time to find the mass of added non-aqueous phase, m_s, by difference. In some cases, separation of the two liquid phases had to be aided by centrifugation using a bench top microcentrifuge (eppendorf mini-spin, Hamburg, Germany) at 5,000 RPM for 2 minutes. A sample of the discoloured indigo reagent was then added to a test tube, taking care to ensure that only aqueous phase was added by disposing of the first few drops from the syringe, and stored in the dark until analysis for not longer than 1 hour. This procedure was repeated at various ozone concentrations in the gas phase ranging between 2.4 g/m^3 NTP (NTP: 0°C, 1 atm) and 70.2 g/m^3 NTP. Once all samples had been collected, their absorbance at 600nm, $(Abs_{600})_f$, was measured using the spectrophotometer. The absorbance of a sample of unreacted IR2, $(Abs_{600})_0$, was also measured in order to determine the decrease in absorbance of the reacted samples. All measurements of absorbance were made relative to a blank of deionised water. The concentration of ozone in each sample of non-aqueous phase was then calculated using Equation 1 assuming that PMX-0245 and vegetable oil are insoluble in water.

$$C_s = \frac{\rho_s}{\rho_{ind}} \frac{m_{ind}}{m_s} \frac{M_{O_3}}{\varepsilon_{ind} L} \left[(Abs_{600})_0 - (Abs_{600})_f \right] \quad [1]$$

where: C_s is non-aqueous phase sample ozone concentration (mg L^{-1}), ρ_s and ρ_{ind} are densities of non-aqueous phase solvent and indigo reagent solution respectively (g L^{-1}), m_{ind} and m_s are respective masses of indigo reagent solution and non-aqueous phase solvent added to syringe (g), M_{O3} is the molecular mass of ozone (48,000 mg/mol), ε_{ind} is the extinction coefficient of indigo (20,069 L mol^{-1} cm^{-1}) L is path length of cell (1 cm), $(Abs_{600})_0$ is absorbance at 600 nm of indigo reagent solution and $(Abs_{600})_f$ is absorbance at 600 nm of the discoloured indigo reagent solution sample.

When analysing for high concentrations of ozone in the non-aqueous phase, IR3 was used instead of IR2 and the volumes of aqueous and non-aqueous phases added to the syringe adjusted accordingly; $(Abs_{600})_f$ was then found by diluting IR3 by a factor of 10, measuring its absorbance and then multiplying this value by 10.

2.1.6 Indigo transfer into the non-aqueous solvent phase

The absorbance of IR2 at 600 nm was measured. Three 25 mL samples of the same indigo solution were mixed with equal volumes of non-aqueous phase at 600 RPM for 5 minutes. The liquids were then transferred to a separating funnel and allowed to settle for 10 minutes prior to measuring the absorbance of the IR2 a second time, additional separation of the liquid phases could be carried out by centrifugation. Any decrease in measured absorbance was assumed to be due to transfer of indigo trisulfonate from the aqueous solution into the non-aqueous phase.

2.1.7 Stability of ozonated indigo solution over 6 hours

A sample of 40 mL IR2 was ozonated by bubbling oxygen containing ozone through the solution with shaking by hand until partial discolouration had occurred, compressed air was then bubbled through the solution to remove any unreacted ozone and the absorbance at 600 nm measured. The solution was then stored in the dark and its absorbance measured once every hour for six hours.

2.1.8 Effect of temperature on indigo solution absorbance

Five samples of 3 mL of IR2 were prepared in test tubes and either cooled or warmed such that 5 samples of different temperatures between 16°C and 36°C were obtained. The

absorbance of each sample at 600 nm was then measured immediately followed by the temperature of the sample using a digital thermometer.

2.1.9 Limits of detection

The lower limit of detection (LLOD) of ozone in the non-aqueous solvent phase was taken to be the equivalent concentration of ozone required to cause the minimum detectable decrease, with reasonable certainty, in absorbance of a sample of indigo solution at 600 nm (IUPAC, 1997). The minimum detectable decrease in absorbance of indigo solution was determined by multiplying the spectrophotometer noise by a factor of three and subtracting this from the initial absorbance of the indigo solution, $(Abs_{600})_0$, for each sample. The noise of the spectrophotometer was determined from the standard deviation of the absorbance at 600 nm of ten samples of deionised water (IUPAC, 1997). The upper limit of detection (ULOD) of ozone in the non-aqueous solvent phase was taken to be the equivalent concentration of ozone required to cause the maximum detectable decrease in absorbance of a sample of indigo solution at 600 nm. The maximum detectable decrease in absorbance of indigo solution was determined as the difference between $(Abs_{600})_0$ and the minimum detectable absorbance of a sample of indigo solution as determined by the positive value of the spectrophotometer noise multiplied by three. Equation [1] was then used to convert the minimum and maximum changes in absorbance to the minimum and maximum detectable ozone concentrations in the non-aqueous solvent.

2.2 Reaction products and stoichiometry

A solution of IR2 was prepared and analysed using an Agilent Technologies 1200 series HPLC system equipped with micro vacuum degasser and autosampler. The column used was a Thermo Scientific Hypersil GOLD 150 mm x 4.6 mm C18 column with particle size of

5 μm. The mobile phase solvents used were: (A) 20 mM ammonium acetate, and (B) HPLC grade methanol. The method was run isocratically at a mobile phase solvent ratio of 90:10 A:B, at a flow rate of 1.000 mL min^{-1} for 15 minutes. The injection volume was 10 μL and the column was thermostated at 25°C. Detection was carried out via diode array detector at wavelengths of 245, 250 and 600 nm. The solution was then partially ozonated by bubbling oxygen containing a low concentration of ozone through the solution for 10 seconds. Any ozone remaining in the solution was then removed by bubbling compressed air through the solution and a second sample was then taken for analysis. The solution was then ozonated further, excess ozone being removed and samples taken for analysis every 10 seconds, until the blue colour of the solution, as detected by visual inspection, had completely disappeared. These samples were then analysed by HPLC in order to observe how the ratio of reactants to products varied with ozonation time. A solution of non-buffered 0.1 mmol L^{-1} potassium indigo trisulfonate was also prepared and the above procedure was applied to this solution in order to assess the role of the phosphate buffer in the IR2 solution.

Calibration standards of 0.04, 0.08, 0.12, 0.16 and 0.20 mmol L^{-1} potassium indigo trisulfonate in acidic solution and isatin-5-sulfonic acid in acidic solution were prepared and analysed via the same HPLC method described above, the latter being a product of the reaction between indigo trisulfonate and ozone (Figure 1) (Bader and Hoigne, 1981). The retention time and peak areas of the peaks generated were then compared in order to determine the stoichiometry of the reaction between indigo trisulfonate in acidic solution and ozone.

3. Results

3.1 Extinction coefficient of indigo ε_{ind}

Figure 4 shows the correlation between absorbance (AU) and concentration of indigo trisulfonate (mol L^{-1}). The slope of the line obtained from the triplicate measurements gave the value of ε_{ind} that was found to be 20,069±412 L Mol^{-1} cm^{-1}. Each of the three experimental runs shows linear correlation and is in close agreement with the other two. The value of ε_{ind} found in this study is close to that obtained by Bader and Hoigné (1981).

3.2 Ozone solubility

For the solvent PMX-0245, a linear correlation was found between ozone concentration in the gas phase (mg L^{-1}) and ozone concentration in the non-aqueous solvent phase (mg L^{-1}) as shown in Figure 5(a). The relative ozone solubility was found to be equal to 1.71±0.09 mg L^{-1} per mg L^{-1} in the gas phase at 25±1.0 $^{\circ}$C.

For vegetable oil, the relationship between solvent phase ozone concentration, C_s, and gas phase ozone concentration, C_g, was found to follow a power law model of the form;

$$C_s = kC_g{}^n \qquad [2]$$

where: concentrations are expressed in mg L^{-1}, the value of k is 0.148 and the value of n is 0.767, this result can be seen in Figure 5(b).

It was also observed that during ozonation of the vegetable oil, the temperature of the oil increased from room temperature up to a maximum of 49.5°C. Besides, the density at room temperature of the vegetable oil also increased from approximately 0.91 g mL^{-1} to 0.93 g mL^{-1}.

3.3 Indigo transfer into non-aqueous solvent phase

It was found that there was no transfer of indigo trisulfonate into either of the non-aqueous phase solvents. Visual inspection of the non-aqueous solvents after mixing detected no

visible colour and spectrophotometric analysis of the indigo solution showed no change in absorbance at 600 nm from its initial values of 2.0033±0.0165 AU for PMX-0245 and 1.9583±0.0021 AU for vegetable oil as shown in Table 1.

3.4 Effect of ozonation on indigo trisulfonate

IR2 solution was observed to contain three distinct compounds with very similar UV-vis spectra eluting from the HPLC column at approximately 2.1, 4.1 and 13 minutes as shown in Figure 6(a) (peaks 1, 2 and 3). After ozonation, the presence of these three indigo derived compounds was not observed and instead two new peaks had appeared (Figure 6(b)) which were caused by the formation of isatin-5-sulfonic acid (peak 5 as determined by a standard) and its predicted corresponding disulfonate (peak 4), thus confirming the assumed chemical reaction between indigo trisulfonate in acidic solution and ozone. Figure 7(a) shows the linear relationship between the absolute value of the change of concentration of indigo solution used in the reaction and the concentration of isatin-5-sulfonic acid produced; the gradient of this plot indicates a stoichiometry of 0.93. The relationship between the production of each of the two isatin-sulfonates was observed to be linear indicating that they are formed in a constant ratio to one another, this is shown Figure 7(b).

3.5 Effect of buffering indigo trisulfonate solution prior to ozonation

It was observed that acidifying the indigo trisulfonate solution with phosphate buffer to make IR2 prior to ozonation acted to slightly increase the retention time of the compounds within the HPLC column in addition to reducing the number of products formed by the reaction of ozone with indigo trisulfonate. Figure 8 shows column retention times for the compounds present in (a) non-ozonated IR2, (b) partially ozonated non-buffered indigo trisulfonate solution and (c) partially ozonated buffered indigo trisulfonate solution. Peak 6 was only

observed to be formed when the indigo trisulfonate solution was not acidified prior to ozonation. The compound responsible for causing peak 6 has not been identified in this study.

3.6 Sample stability over 6 hour time period

Absorbance at 600 nm of IR2 after initial ozonation was observed to decrease linearly over a 6 hour time period by approximately 15 % however the decrease after one hour from initial ozonation was less than 5 % of the initial value.

3.7 Effect of temperature on absorbance

An increase in temperature from 16±1°C to 36±1°C of IR2 solution was accompanied by a decrease in absorbance at 600 nm of less than 1 %. Hence the effect of temperature was neglected during measurement of indigo concentration by UV/Vis spectrophotometer.

3.8 Limits of detection and precision of the procedure

The limit of detection of ozone in non-aqueous phase was found to be 2.6 $\mu g\ L^{-1}$ whilst the maximum was 142.7 $mg\ L^{-1}$.

The analytical procedure for analysing the non-aqueous phase ozone concentration was found to be very precise due to the nature of the equipment used, with percentage errors in measurements of absorbance and mass rarely greater than 0.01 %. If spectrophotometric determination of the absorbance of reacted indigo solution is carried out immediately after the discolouration reaction then the percentage error will be less than 1 %.

The largest errors present in this investigation are expected to have arisen during ozonation of the non-aqueous phase and are estimated to be approximately 5 % if spectrophotometric analysis is carried out soon after the discolouration reaction occurs.

4. Discussion

4.1 Relative ozone solubility in PMX-0245

Henry's law predicts that ozone solubility in PMX-0245 should increase linearly with ozone concentration in the gas phase as confirmed by Ward et al. (2003). The linearity of the data as shown in Figure 5(a) confirm Henry's law, which indicates the suitability of the experimental procedure followed in measuring dissolved ozone concentration in PMX-0245.

4.2 Ozonation of vegetable oil

The observed increase in temperature of the vegetable oil over the course of ozonation, in addition to the increase in measured density of the vegetable oil, suggests that some reaction had occurred between the vegetable oil and the ozone, altering the chemistry of the vegetable oil used. The reaction of ozone with unsaturated fatty acids such as vegetable oils and sunflower oils is well documented and suggests that the ozone becomes incorporated into the molecular structure of the oil by bonding at any C=C double bonds to form Criegee ozonides (Criegee, 1975); this explains the exothermic behaviour observed and could also account at least partially for the increased density. Previous studies also suggest that an increase in viscosity would also be observed had this characteristic of the oil been measured (Diaz et al., 2001; Tellez et al., 2006; Sega et al., 2010). The fact that ozone appears to react with the vegetable oil used in this experiment by attacking the C=C double bonds suggests that the concentration of ozone in vegetable oil will change over the course of ozonation as a function of the number of C=C double bonds still present in the vegetable oil. It is important therefore to realise that the power law type correlation observed for the vegetable oil relative ozone solubility is only representative of ozone concentration in vegetable oil after ozonation for 1 hour and that different correlations can be expected if the ozonation time were to change.

4.3 Extinction coefficient

This investigation has shown that there exists some uncertainty in the value of ε_{ind} for potassium indigo trisulfonate; the value was found to be 20,069±412 L mol^{-1} cm^{-1}, this value is close to that initially detailed by Bader and Hoigné (1981) of 20,000 L mol^{-1} cm^{-1} but significantly lower than that reported by Chiou et al. (1995) of 23,150±80 L mol^{-1} cm^{-1}. It is hence recommended that this value should be independently determined by any laboratory using this procedure with each batch of potassium indigo trisulfonate purchased.

4.4 Indigo transfer into non-aqueous solvent phase

No transfer of indigo trisulfonate into either of the non-aqueous phase solvents was observed. This indicates that the discolouration reaction between ozone and indigo trisulfonate in acidic solution occurred only in the aqueous phase and therefore that dissolved ozone from the non-aqueous solvent can be readily transferred from the non-aqueous solvent into the aqueous solvent.

4.5 Stability of method over 6 hours

This investigation suggests that after initial ozonation of the indigo solution, the absorbance at 600 nm will continue to fall steadily for a period of up to 6 hours, this is contrary to the findings by Bader and Hoigné (1981). The observed decrease was quite gradual with a decrease in the first hour after ozonation of less than 5 %, this suggests therefore that the measurements of absorbance at 600 nm of samples analysed shortly after being discoloured will not contain significant errors.

4.6 Effect of tempereature on absorbance

The temperature of indigo reagent was found to have almost no effect on its measured absorbance at 600 nm implying that the temperature at which spectrophotometric measurements are made does not need to be controlled in order to achieve reproducible results.

4.7 Notes on the use of gastight syringes

The use of gastight syringes as reaction vessels was found to make a significant improvement to the results over those collected by mixing the indigo reagent and non-aqueous solvent samples in an open vessel and their use is recommended for any future work involving the indigo method for solvents immiscible with water. Samples of non-aqueous phase analysed in this investigation were of a much smaller volume than those water samples for which the indigo method was initially intended, in addition to the dissolved ozone concentration being much higher, hence significant care had to be taken so as to ensure complete discolouration of the indigo reagent solutions did not occur, relatively conservative volumes of non-aqueous phase of below 0.8 mL were in some cases required in order to avoid this.

4.8 Limit of detection and precision of the procedure

The procedure was found to be effective at analysing ozone concentration in non-aqueous phase down to a lower limit of detection of 2.6 $\mu g\,L^{-1}$. The relatively low volume of non-aqueous phase sample, when compared with the volumes of aqueous sample used by Bader and Hoigné (1981), and the fact that no dilution of the indigo solution occurs on addition to the sample, unlike when aqueous samples are analysed, allows this procedure to be used for the quantification of ozone in non-aqueous phase samples at concentrations up to an upper limit of detection of 142.7 mg L^{-1}.

The greatest uncertainty in this study arose from the fact that ozone concentration in the gas phase could not be recorded during the ozonation procedure, only before and after. The greatest care was taken to ensure that the ozone generator had reached a steady state of operation prior to ozonation but differences between the readings before and after give rise to some uncertainty in these values. The error caused by this is estimated to be approximately 5 % and this is in agreement with the spread of results as indicated in Figure 5(a).

The analytical procedure itself was found to have very low errors due to the precision of the equipment used. The use of a four figure analytical balance to measure the quantity of each liquid phase inside the syringe, instead of using the scale on the syringe barrel likely helped reduce the associated error significantly. It is estimated that if spectrophotometric analysis of the discoloured indigo solution is carried out immediately after reaction and separation of the two liquid phases, then the overall percentage error associated with the new indigo method is less than 1 %.

4.9 Confirmation of stoichiometry of reaction

Kettle et al. (2004) investigated the reaction mechanism between indigo carmine and ozone and proposed the mechanism of one molecule of indigo carmine and one molecule of ozone forming two molecules of isatin-5-sulfonic acid, previous works have assumed the same stoichiometry in the reaction of indigo trisulfonate in acidic solution with ozone as a means of measuring dissolved ozone concentration in aqueous solutions (Bader and Hoigne, 1982; Chiou et al., 1995). HPLC analysis of the reaction products was carried out in order to check this stoichiometry. The five peaks shown in Figure 6 (a & b) are caused by compounds involved in the indigo reaction with ozone, which made the quantification of reaction products possible. Peak 2 was assumed to have been caused by indigo trisulfonate from the original potassium indigo trisulfonate powder used in this investigation as it was the largest

of the three peaks caused by this solution, peak 3 was identified as indigo carmine by comparing its UV-vis spectrum, as determined by the HPLC diode array detector, with that from the literature (Gomes et al., 2003; Torres-Martinez et al., 2012) and peak 1 was not identified but was assumed to be a third indigo derived compound due to the similarities in its UV-vis spectrum to those of indigo trisulfonate and indigo carmine. After ozonation to complete bleaching, the presence of only two compounds was observed; peak 5 was confirmed to be isatin-5-sulfonic acid by comparison with a standard and peak 4 was assumed to be the predicted corresponding isatin-disulfonic acid, this however could not be confirmed experimentally due to the commercial unavailability of this compound.

Isatin-5-sulfonic acid concentration (mmol L^{-1}) was observed to increase linearly with respect to an increase in reacted indigo trisulfonate (mmol L^{-1}) as seen in Figure 7(a), this served as a confirmation of the stoichiometric ratio of the reaction between ozone and indigo trisulfonate of approximately 0.93. This value is less than the value suggested in the literature (i.e. 1.0) by about 7% (Bader and Hoigne, 1981; Kettle et al., 2004), possibly due to side reactions between ozone and the other compounds (peaks 1 and 3 – Figure 6(a)) and systematic errors. In addition to this, peak areas of the peaks attributed to isatin-disulfonic acid and isatin-5-sulfonic acid were observed to increase in a constant ratio to one another as ozonation time was increased (Figure 7(b)); this serves as secondary confirmation of the proposed reaction between indigo trisulfonate in acidic solution and ozone. It is important to note that this reaction stoichiometry was only observed with indigo trisulfonate in acidic solution buffered to pH 1.80. A non-buffered solution of indigo trisulfonate was found to not follow the same reaction stoichiometry and additional reaction products were formed. This is in agreement with previous work carried out by Bader and Hoigné (1981) who found that in

order for the reaction between ozone and indigo trisulfonate to proceed following the above mentioned stoichiometry, the pH of the indigo solution should be kept below 4.

5. Conclusions

It is concluded that this investigation has sufficiently demonstrated an adapted indigo method for the analysis of ozone dissolved in non-aqueous liquid phases in addition to confirmation that the reaction between indigo trisulfonate and ozone has a stoichiometric ratio of about one, and hence that the proposed method is suitable for the analysis of dissolved ozone in non-aqueous phases. The use of gastight syringes inert to ozone for the analysis of dissolved ozone via the indigo method in any future works is also highly recommended so as to avoid loss of ozone from liquid samples via off gassing during liquid transfer.

6. References

Bader, H., and J. Hoigne, "Determination of ozone in water by the indigo method", Water Research 15(4): 449-456 (1981)

Bader, H., and J. Hoigne, "Determination of ozone in water by the indigo method - A submitted standard method", Ozone-Science & Engineering 4(4): 169-176 (1982)

Bhattacharyya, D., T.F. Van Dierdonck, S.D. West, and A.R. Freshour, "Two-phase ozonation of chlorinated organics", Journal of Hazardous Materials 41(1): 73-93 (1995)

Bocci, V., Oxygen-ozone therapy: A critical evaluation Springer, 2002)

Chang, C.Y., and J.N. Chen, "Ozonolysis of 2,4-dichlorophenol in a 2-phase solvent water-system", Water Sci Technol 29(9): 343-346 (1994)

Chiou, C.F., B.J. Marinas, and J.Q. Adams, "Modified indigo method for gaseous and aqueous ozone analysis", Ozone-Science & Engineering 17(3): 329-344 (1995)

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton, Standard methods for the examination of water and wastewater. American Public Health Board, American Water Works Association, Water Environment Federation, Washington, DC (1998).

Collins, A.G., M.R. Farvardin, and Z. Shen, "An alternative approach to gas-phase ozone determination", Ozone-Science & Engineering 11(1): 115-125 (1989)

Criegee, R., "Mechanism of ozonolysis", Angew Chem-Int Edit Engl 14(11): 745-752 (1975)

Diaz, M., I. Lezcano, J. Molerio, and F. Hernandez, "Spectroscopic characterization of ozonides with biological activity", Ozone-Science & Engineering 23(1): 35-40 (2001)

Freshour, A.R., S. Mawhinney, and D. Bhattacharyya, "Two-phase ozonation of hazardous organics in single and multicomponent systems", Water Research 30(9): 1949-1958 (1996)

Gomes, R.L., M.D. Scrimshaw, and J.N. Lester, "Determination of endocrine disrupters in sewage treatment and receiving waters", Trac-Trends in Analytical Chemistry 22(10): 697-707 (2003)

Gottschalk, C., J.A. Libra, and A. Saupe, Ozonation of water & wastewater: A practical guide to understanding ozone and its applications (Weinheim: Wiley-VCH, 2010)

Gromadzka, K., and J. Nawrocki, "Degradation of diclofenac and clofibric acid using ozone-loaded perfluorinated solvent", Ozone-Science & Engineering 28(2): 85-94 (2006)

Guha, A.K., P.V. Shanbhag, K.K. Sirkar, D.A. Vaccari, and D.H. Trivedi, "Multiphase ozonolysis of organics in waste-water by a novel membrane reactor", Aiche J 41(8): 1998-2012 (1995)

Hart, E.J., K. Sehested, and J. Holcman, "Molar absorptivities of ultraviolet and visible bands of ozone in aqueous-solutions", Analytical Chemistry 55(1): 46-49 (1983)

IUPAC, Compendium of chemical terminology, in the "Gold Book", ed by McNaught A.D. and A. Wilkindon. Blackwell Scientific Publications, Oxford (1997).

Kettle, A.J., B.M. Clark, and C.C. Winterbourn, "Superoxide converts indigo carmine to isatin sulfonic acid - Implications for the hypothesis that neutrophils produce ozone", Journal of Biological Chemistry 279(18): 18521-18525 (2004)

Kim, H.S., S.U. Noh, Y.W. Han, K.M. Kim, H. Kang, H.O. Kim, and Y.M. Park, "Therapeutic effects of topical application of ozone on acute cutaneous wound healing", J Korean Med Sci 24(3): 368-374 (2009)

Rakness, K.L., Ozone in drinking water treatment: process design, operation, and optimization (Denver, CO: American Water Works Association, 2005)

Sega, A., I. Zanardi, L. Chiasserini, A. Gabbrielli, V. Bocci, and V. Travagli, "Properties of sesame oil by detailed H-1 and C-13 NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements", Chem Phys Lipids 163(2): 148-156 (2010)

Tellez, G.M., O.L. Lozano, and M.F.D. Gomez, "Measurement of peroxidic species in ozonized sunflower oil", Ozone-Science & Engineering 28(3): 181-185 (2006)

Torres-Martinez, L.M., M.A. Ruiz-Gomez, M.Z. Figueroa-Torres, I. Juarez-Ramirez, and E. Moctezuma, "Sm2FeTaO7 Photocatalyst for Degradation of Indigo Carmine Dye under Solar Light Irradiation", International Journal of Photoenergy: (2012)

Travagli, V., I. Zanardi, G. Valacchi, and V. Bocci, "Ozone and ozonated oils in skin diseases: a review", Mediators of inflammation 2010(610418 (2010)

Ward, D.B., C. Tizaoui, and M.J. Slater, "Ozone-loaded solvents for use in water treatment", Ozone-Science & Engineering 25(6): 485-495 (2003)

Ward, D.B., C. Tizaoui, and M.J. Slater, "Extraction and destruction of organics in wastewater using ozone-loaded solvent", Ozone-Science & Engineering 26(5): 475-486 (2004)

Ward, D.B., C. Tizaoui, and M.J. Slater, "Continuous extraction and destruction of chloro-organics in wastewater using ozone-loaded Volasil (TM) 245 solvent", Journal of Hazardous Materials 125(1-3): 65-79 (2005)

Ward, D.B., C. Tizaoui, and M.J. Slater, "Wastewater dye destruction using ozone-loaded Volasil (TM) 245 in a continuous flow liquid-liquid/ozone system", Chemical Engineering and Processing 45(2): 124-139 (2006)

Tables

Table 1. Change in absorbance at 600 nm of acidified indigo trisulfonate caused by transfer of indigo into solvent phase (initial indigo concentration = 0.1 mmol L^{-1}, contact time = 15 min, T=25±1.0 °C).

PMX-0245		**Vegetable oil**	
Absorbance before contact with solvent (AU)	Absorbance after contact with solvent (AU)	Absorbance before contact with solvent (AU)	Absorbance after contact with solvent (AU)
2.0008	2.0254	1.9608	1.9547
1.9995	1.9946	1.9583	1.9589
1.9997	1.9996	1.9595	1.9573

Figures

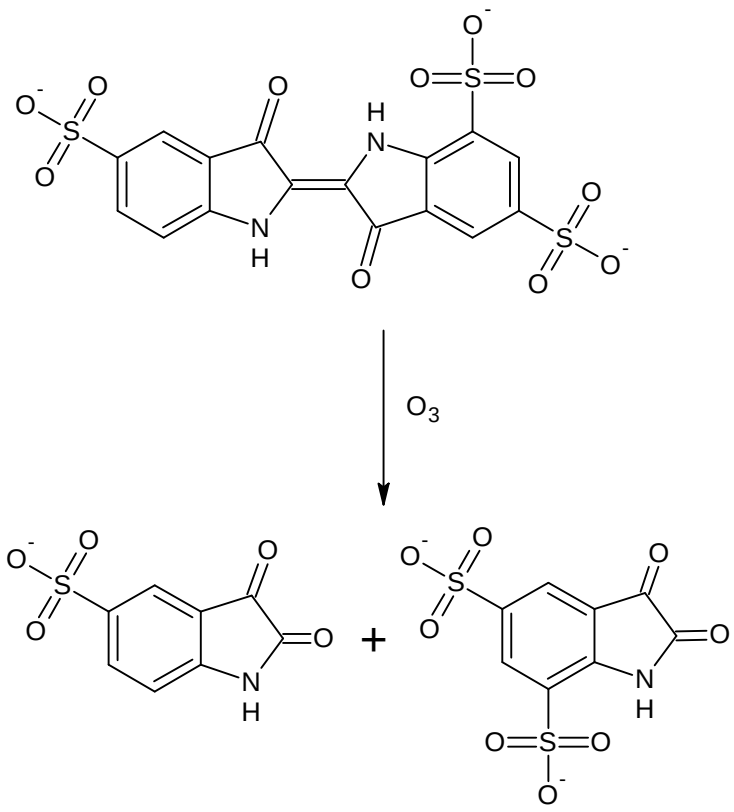


Figure 1. Ozonolysis of indigo trisulfonate to form isatin-5-sulfonic acid and isatin-disulfonic acid.

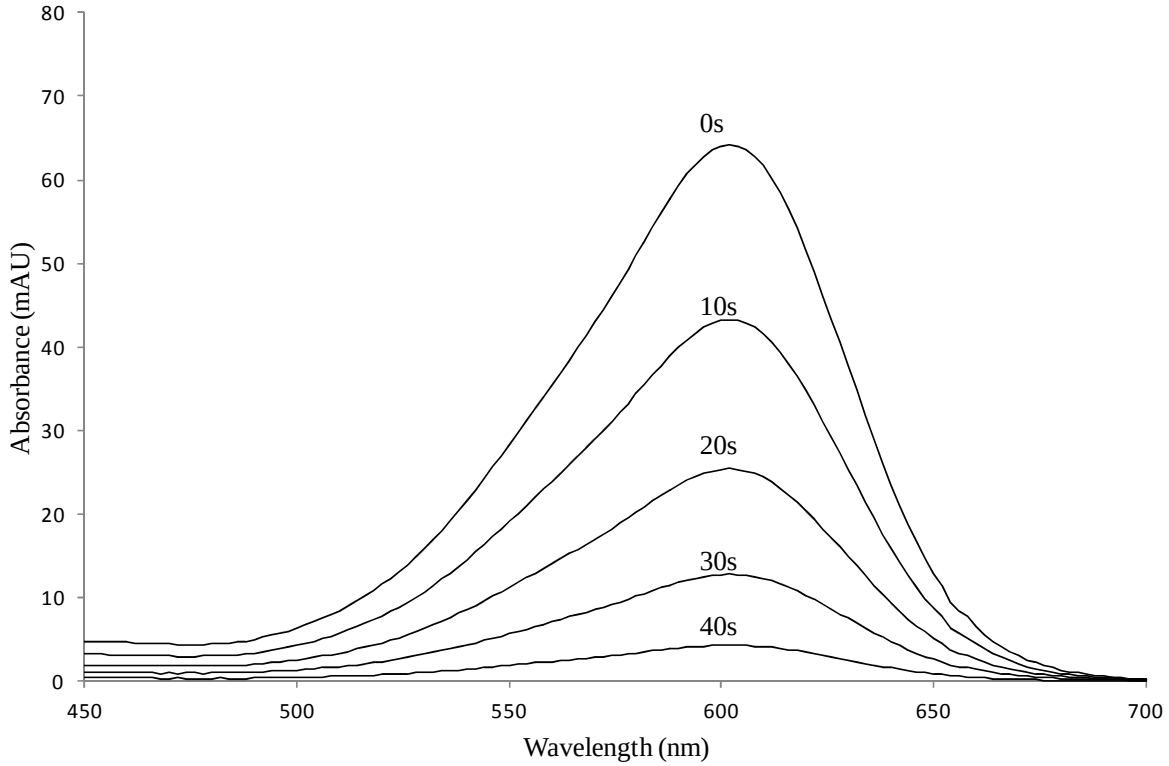


Figure 2. Absorbance spectra of Indigo Reagent II as a function of ozonation time in seconds.

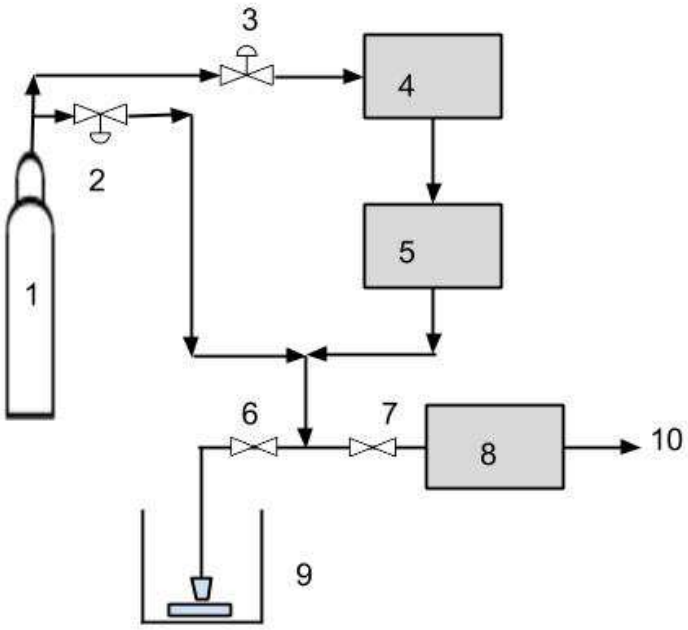
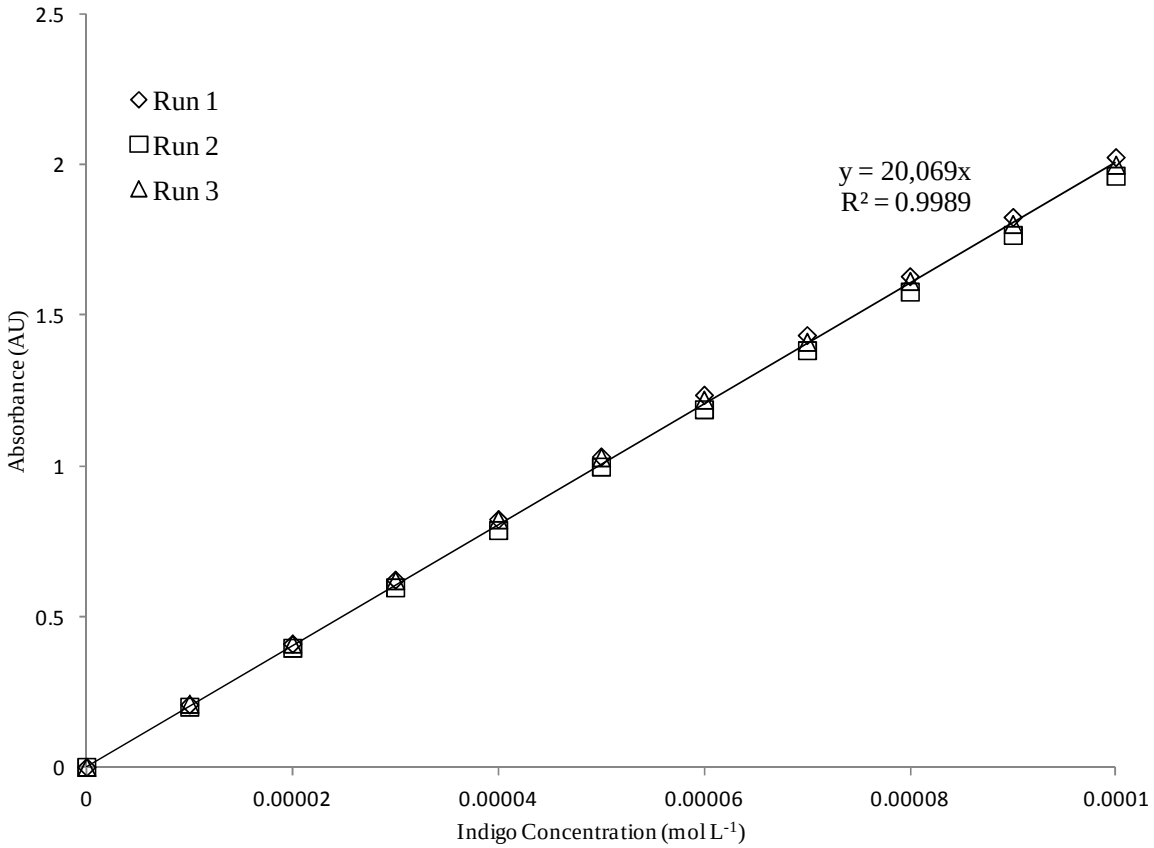


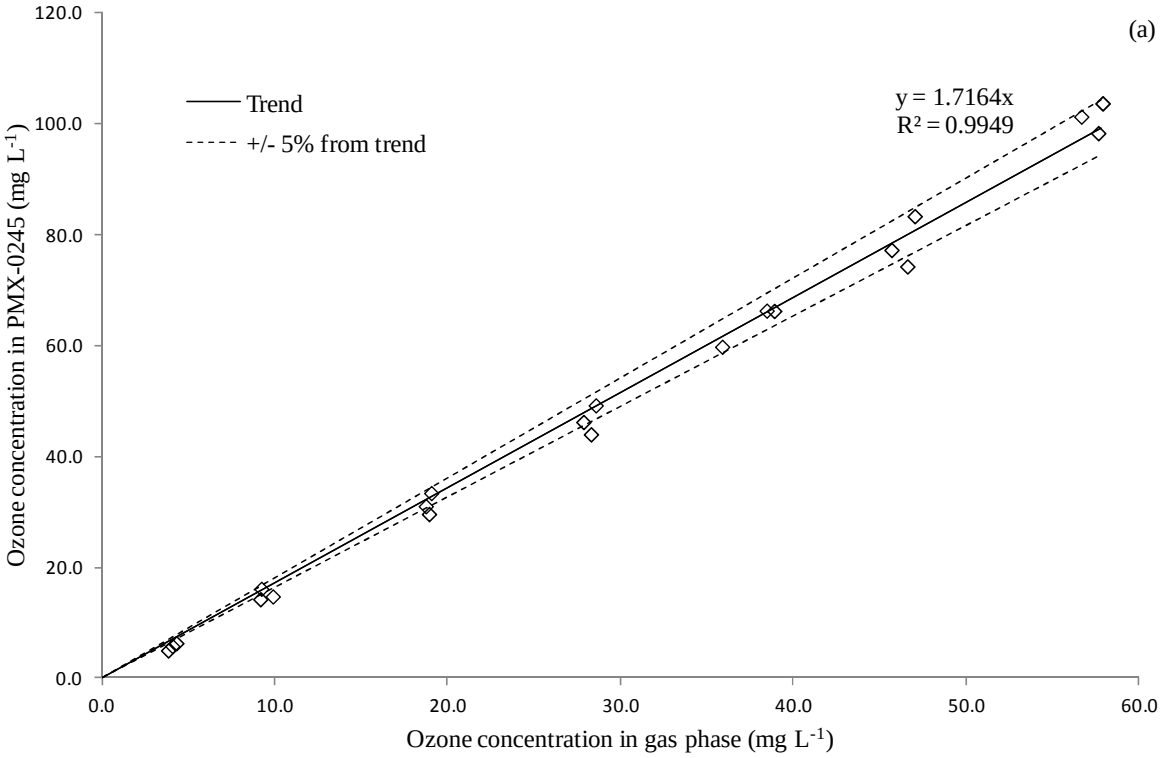
Figure 3. Apparatus for analysis of dissolved ozone: (1) compressed oxygen cylinder, (2, 3) flow control valve, (4) electronic flow meter, (5) ozone generator, (6, 7) valve, (8) ozone analyser, (9) beaker with submerged diffuser and magnestic stirrer, (10) vent through an ozone destructor.



1

2 Figure 4. Correlation between absorbance and concentration of potassium indigo trisulfonate,

3 used for the determination of ε_{ind}.



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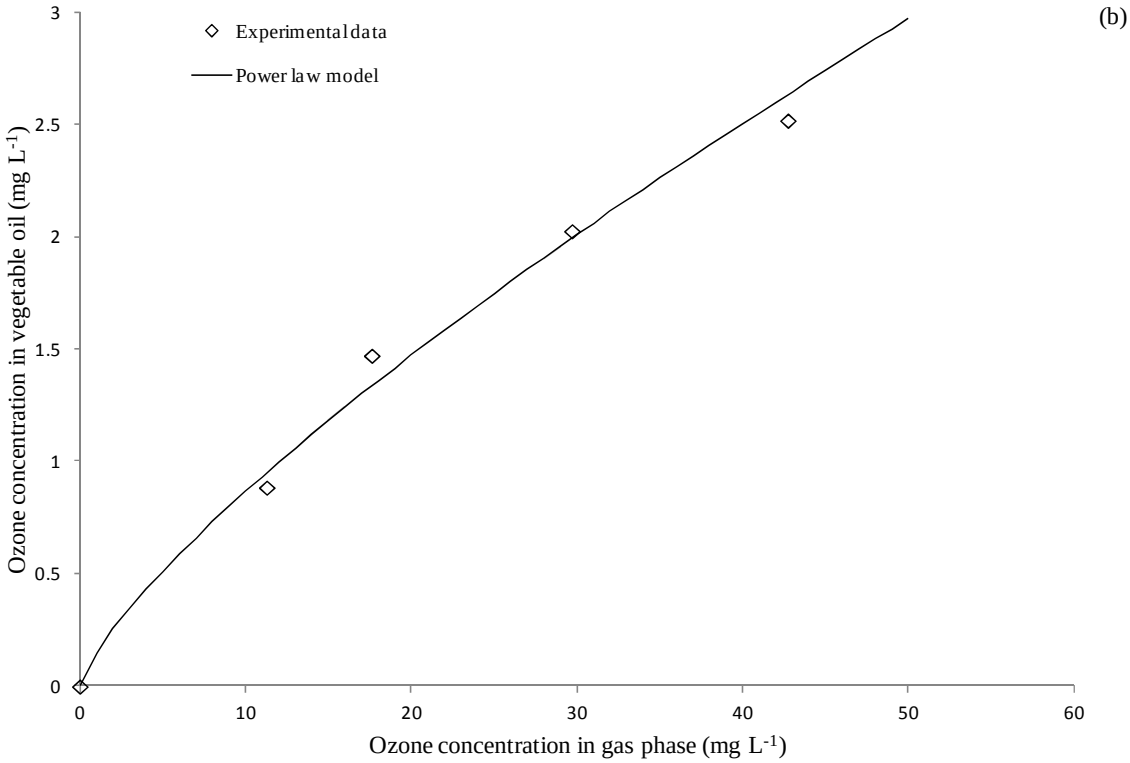


2

3

4 Figure 5. Correlation between ozone concentration in the gas phase and ozone concentration

5 in (a) PMX-0245 (C_s=1.716C_g), (b) vegetable oil (C_s=0.148$C_g^{0.767}$) (T=25 ±1°C).

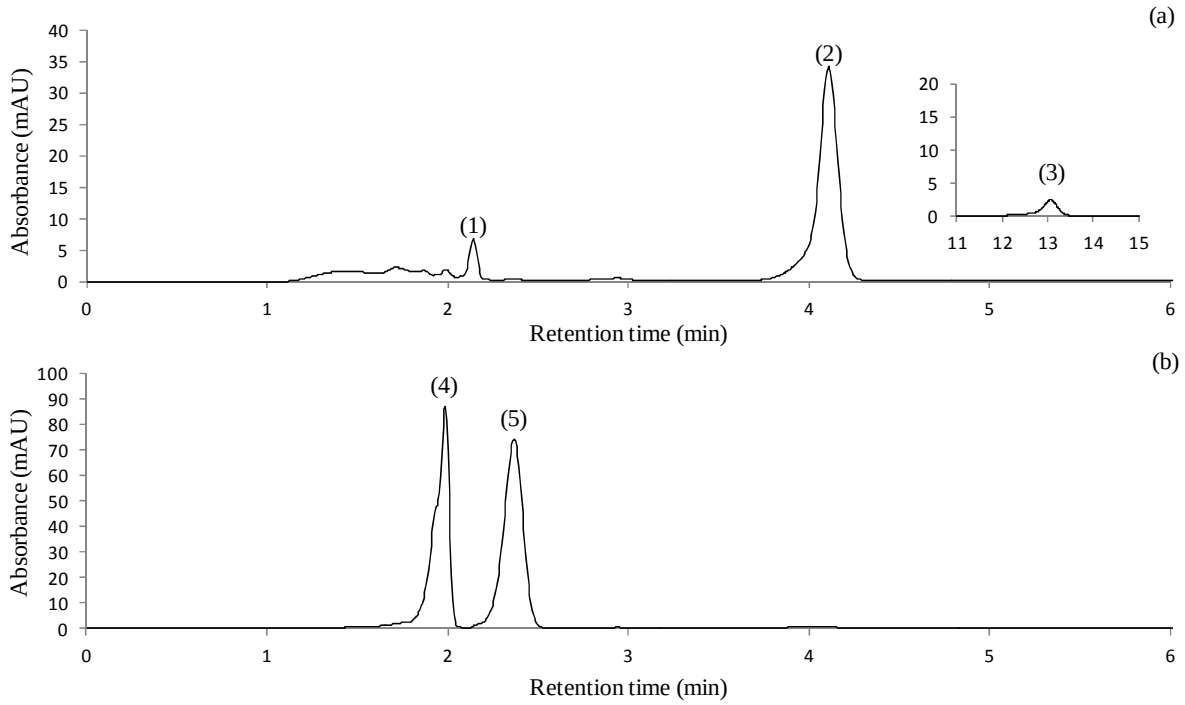
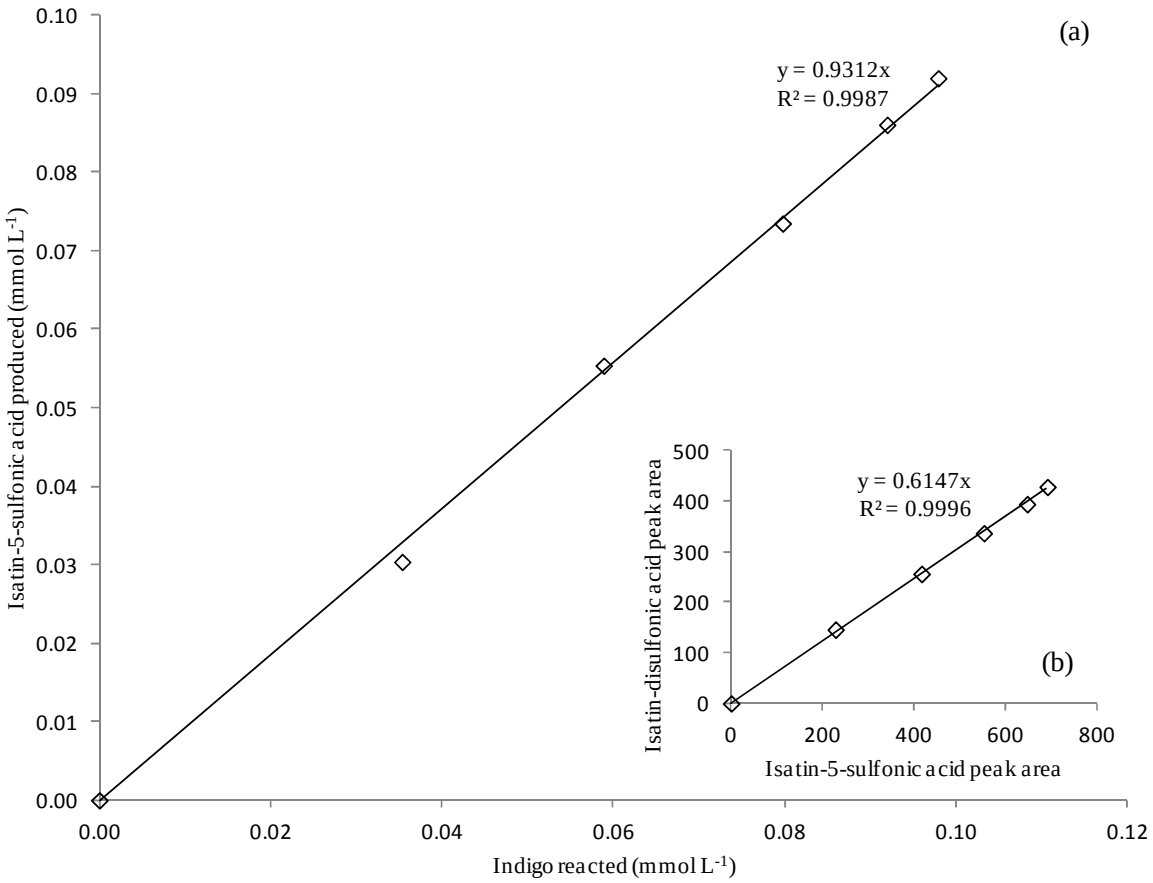


Figure 6. Peaks detected at 250 nm. (a) Indigo Reagent II solution before ozonation, (b) Indigo Reagent II solution after complete ozonation. (Peak 1) unidentified indigo derived compound, (2) Indigo trisulfonate, (3) indigo carmine, (4) isatin-disulfonic acid (5) isatin-5-sulfonic acid.



1

2 Figure 7. (a) linear relationship between the amount of isatin-5-sulfonic acid formed and
3 indigo reacted , (b) linear relationship between HPLC response of isatin-5-sulfonic acid and
4 isatin-disulfonic acid

5

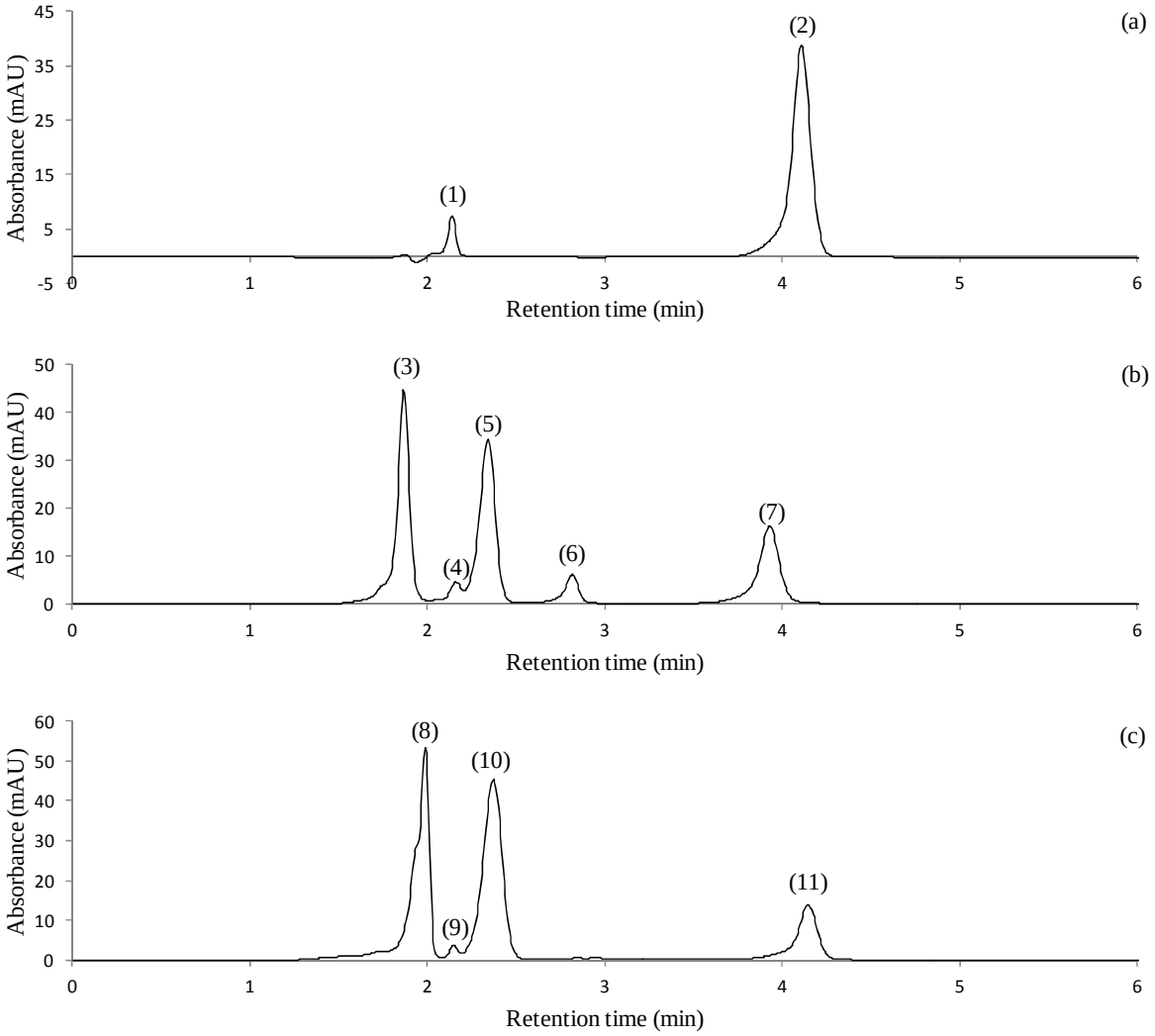


Figure 8. Peaks detected at 600 nm. (a) unreacted Indigo Reagent II, (b) partially ozonated non-buffered indigo trisulfonate solution, (c) partially ozonated buffered indigo trisulfonate solution. (Peak 1, 4, 9) unidentified indigo derived compounds, (2, 7, 11) indigo trisulfonate, (3, 8) isatin-disulfonic acid, (5, 10) isatin-5-sulfonic acid, (6) unidentified reaction product.